CLAIMS

1. A method for typing of HLA class I alleles comprising the following steps from (a) to (d);

(a) A step, using HLA class I gene or nucleic acids containing their fragment for a template,

(1) To non-selectively amplify all HLA-A alleles, all HLA-B alleles or all HLA-C alleles by a PCR method using a primer pair which can amplify all the HLA-A alleles, all the HLA-B alleles or all the HLA-C alleles, or

(2) To selectively amplify a specific group consisting of specific HLA-A alleles or specific HLA-B alleles by a PCR method using a primer pair which is specific to the common sequence to alleles of the specific group consisting of the specific HLA-A alleles or the specific HLA-B alleles,

(b) A step to add the above products amplified by the PCR method to wells of microtiter plates, wherein each well is modified with a carboxyl group to covalently immobilize amino-modified DNA probes which can specifically hybridize with the sequence of at least one specific HLA-A allele, at least one specific HLA-B allele or at least one specific HLA-C allele, and to hybridize the amplified products with the immobilized DNA probes, wherein the DNA probes are selected depending on the above amplified specific HLA class I gene or group;

(c) A step to detect as signals whether or not the amplified products are hybridized with the immobilized probes; and

(d) A step to determine the type of the HLA class I allele based on the signal pattern detected at the step (c) according to the Typing Table.

2. The method for typing of the HLA class I alleles claimed in claim 1,

wherein at least one of the primer pair is labeled.

3. The method for typing of the HLA class I alleles claimed in claim 2, which comprises hybridizing the amplified products obtained by the PCR method with the immobilized DNA probes, adding an enzyme-conjugate which specifically bonds to the label of the amplified products thereto at the same time as or after the hybridization, and adding a chromogenic substrate, a luminescent substrate or a fluorescent substrate to the mixture, to detect as signals whether or not the amplified products are hybridized with the immobilized DNA probes.

4. The method for typing of the HLA class I alleles claimed in claim 3, wherein at least one of the primer pair is biotinylated and the enzyme-conjugate which specifically bonds to the label of the amplified products obtained by the PCR method is an enzyme-conjugated streptavidin.

5. The method for typing of the HLA class I alleles claimed in any one of claims 1 to 4, wherein the hybridization of the amplified products obtained by the PCR method with the immobilized DNA probes is performed in a solution containing formamide.

6. The method for typing of the HLA class I alleles claimed in claim 5, wherein the reaction temperature for hybridization of the amplified products obtained by the PCR method with immobilized DNA probes is about 37°C.

7. The method for typing of the HLA class I alleles claimed in claims 5 or 6, wherein the temperature for washing after hybridization of the amplified products by the PCR method with the immobilized DNA probes and/or after the binding reaction of the label of the amplified products with the enzyme-conjugate is room temperature.

8. The method for typing of the HLA class I alleles claimed in any one of claims 1 to 7, wherein the amino-modified DNA probe which can specifically

hybridize with at least one specific HLA-A allele, at least one specific HLA-B allele or at least one specific HLA-C allele, is selected from the group consisting of A98T (SEQ ID No.:1), A98A (SEQ ID No.:2), A160A (SEQ ID No.:3), A239A (SEQ ID No.:4),A238A (SEQ ID No.:5), A240T (SEQ ID No.:6), A257TC (SEQ ID No.:7), A259AC (SEQ ID No.:8), A270T (SEQ ID No.:9), A282C (SEQ ID No.:10), A290T (SEQ ID No.:11), A299T (SEQ ID No.:12), A302G (SEQ ID No.:13), A355G (SEQ ID No.:14), A362TA (SEQ ID No.:15), A362TT (SEQ ID No.:16), A368A (SEQ ID No.:17), A368G (SEQ ID No.:18), A368T (SEQ ID No.:19), A402G (SEQ ID No.:20), A423T (SEQ ID No.:21), A448C (SEQ ID No.:22), A485A (SEQ ID No.:23), A524G (SEQ ID No.:24), A526T (SEQ ID No.:25), A527A (SEQ ID No.:26), A538CG (SEQ ID No.:27), A539A (SEQ ID No.:28), A539T (SEQ ID No.:29), A555T (SEQ ID No.:30), A559G (SEQ ID No.:31), A570CG (SEQ ID No.:32), A570GT (SEQ ID No.:33), A779A (SEQ ID No.:34), A843A (SEQ ID No.:35), BL1 (SEQ ID No.:36), BL3 (SEQ ID No.:37), BL4 (SEQ ID No.:38), BL5 (SEQ ID No.:39), BL9 (SEQ ID No.:40), BL10 (SEQ ID No.:41), BL11 (SEQ ID No.:42), BL24 (SEQ ID No.:43), BL25 (SEQ ID No.:44), BL34 (SEQ ID No.:45), BL35 (SEQ ID No.:46), BL36 (SEQ ID No.:47), BL37 (SEQ ID No.:48), BL38 (SEQ ID No.:49), BL39 (SEQ ID No.:50), BL40 (SEQ ID No.:51), BL41 (SEQ ID No.:52), BL42 (SEQ ID No.:53), BL56 (SEQ ID No.:54), BL57 (SEQ ID No.:55), BL78 (SEQ ID No.:56), BL79 (SEQ ID No.:57), BL222A (SEQ ID No.:58), BL272GA (SEQ ID No.:59), BL226G (SEQ ID No.:60), BL292G (SEQ ID No.:61), BL292T (SEQ ID No.:62), BL361G (SEQ ID No.:63), BL409T (SEQ ID No.:64), BL512T (SEQ ID No.:65), BL538CG (SEQ ID No.:66), BL538G (SEQ ID No.:67), CC (SEQ ID No.:68), A-12 (SEQ ID No.:69), A-2 (SEQ ID No.:70), A-3 (SEQ ID No.:71), A-4 (SEQ ID No.:72), A-54 (SEQ ID No.:73), B-1 (SEQ ID No.:74), B-2 (SEQ ID No.:75), C-12 (SEQ ID No.:76), C-24 (SEQ ID No.:77), C-33 (SEQ ID No.:78), C-43 (SEQ ID No.:79), 134-g (SEQ ID No.:80), 134-A2 (SEQ ID No.:81), 353TCA1 (SEQ ID No.:82), 343A (SEQ ID No.:83), A34 (SEQ ID No.:100), A282CT

(SEQ ID No.:101), A290TR (SEQ ID No.:102), A302GR (SEQ ID No.:103), A414A (SEQ ID No.:104), A468T (SEQ ID No.:105), A489A (SEQ ID No.:106), A502C (SEQ ID No.:107), A538TG (SEQ ID No.:108), BL39R (SEQ ID No.:109), BL50 (SEQ ID No.:110), BL77 (SEQ ID No.:111), BL272A (SEQ ID No.:112), BL263T (SEQ ID No.:113), BL527A (SEQ ID No.:114), BL570GT (SEQ ID No.:115), RA-2 (SEQ ID No.:116), RA-41 (SEQ ID No.:117), RB-28 (SEQ ID No.:118), 201g1 (SEQ ID No.:119), C206gR (SEQ ID No.:120), R341A (SEQ ID No.:121), R343g3 (SEQ ID No.:122), 353TCC (SEQ ID No.:123), 361T1 (SEQ ID No.:124), 361T368g (SEQ ID No.:125), 361T368T1 (SEQ ID No.:126), 369C (SEQ ID No.:127), 387g1 (SEQ ID No.:128), 526AC2 (SEQ ID No.:129), 538gAC (SEQ ID No.:130), complementary strands thereof and nucleic acids which comprises one to several bases are deleted from or added to the end of them.

9. The method for typing of the HLA class I alleles claimed in any one of claims 1 to 8, which comprises primers capable of amplifing all the HLA-A alleles, all the HLA-B alleles or all the HLA-C alleles, or primers specific to the common sequence to alleles of the specific group consisting of the specific HLA-A alleles or the specific HLA-B alleles, is selected from A2-5T (SEQ ID No.:84), A3-273T (SEQ ID No.:85), A4-8C (SEQ ID No.:86), A4-254G (SEQ ID No.:87), BASF-1 (SEQ ID No.:88), BASR-1 (SEQ ID No.:89), CGA011 (SEQ ID No.:90), CGA012 (SEQ ID No.:91), AIn3-66C (SEQ ID No.:92), 5BCIn37-34C (SEQ ID No.:96), 5BCIn37-24g (SEQ ID No.:97) and 5BCIn37-34g2 (SEQ ID No.:99).

10. A DNA probe used for a typing method of the HLA class I alleles, which is selected from the group consisting of A98T (SEQ ID No.:1), A98A (SEQ ID No.:2), A160A (SEQ ID No.:3), A239A (SEQ ID No.:4), A238A (SEQ ID No.:5), A240T (SEQ ID No.:6), A257TC (SEQ ID No.:7), A259AC (SEQ ID No.:8), A270T (SEQ ID No.:9), A282C (SEQ ID No.:10), A290T (SEQ ID No.:11), A299T (SEQ ID No.:12), A302G (SEQ ID No.:13), A355G (SEQ ID No.:14), A362TA (SEQ ID No.:15),

A362TT (SEQ ID No.:16), A368A (SEQ ID No.:17), A368G (SEQ ID No.:18), A368T (SEQ ID No.:19), A402G (SEQ ID No.:20), A423T (SEQ ID No.:21), A448C (SEQ ID No.:22), A485A (SEQ ID No.:23), A524G (SEQ ID No.:24), A526T (SEQ ID No.:25), A527A (SEQ ID No.:26), A538CG (SEQ ID No.:27), A539A (SEQ ID No.:28), A539T (SEQ ID No.:29), A555T (SEQ ID No.:30), A559G (SEQ ID No.:31), A570CG (SEQ ID No.:32), A570GT (SEQ ID No.:33), A779A (SEQ ID No.:34), A843A (SEQ ID No.:35), BL1 (SEQ ID No.:36), BL3 (SEQ ID No.:37), BL4 (SEQ ID No.:38), BL5 (SEQ ID No.:39), BL9 (SEQ ID No.:40), BL10 (SEQ ID No.:41), BL11 (SEQ ID No.:42), BL24 (SEQ ID No.:43), BL25 (SEQ ID No.:44), BL34 (SEQ ID No.:45), BL35 (SEQ ID No.:46), BL36 (SEQ ID No.:47), BL37 (SEQ ID No.:48), BL38 (SEQ ID No.:49), BL39 (SEQ ID No.:50), BL40 (SEQ ID No.:51), BL41 (SEQ ID No.:52), BL42 (SEQ ID No.:53), BL56 (SEQ ID No.:54), BL57 (SEQ ID No.:55), BL78 (SEQ ID No.:56), BL79 (SEQ ID No.:57), BL222A (SEQ ID No.:58), BL272GA (SEQ ID No.:59), BL226G (SEQ ID No.:60), BL292G (SEQ ID No.:61), BL292T (SEQ ID No.:62), BL361G (SEQ ID No.:63), BL409T (SEQ ID No.:64), BL512T (SEQ ID No.:65), BL538CG (SEQ ID No.:66), BL538G (SEQ ID No.:67), CC (SEQ ID No.:68), A-12 (SEQ ID No.:69), A-2 (SEQ ID No.:70), A-3 (SEQ ID No.:71), A-4 (SEQ ID No.:72), A-54 (SEQ ID No.:73), B-1 (SEQ ID No.:74), B-2 (SEQ ID No.:75), C-12 (SEQ ID No.:76), C-24 (SEQ ID No.:77), C-33 (SEQ ID No.:78), C-43 (SEQ ID No.:79), 134-g (SEQ ID No.:80), 134-A2 (SEQ ID No.:81), 353TCA1 (SEQ ID No.:82), 343A (SEQ ID No.:83), A34 (SEQ ID No.:100), A282CT (SEQ ID No.:101), A290TR (SEQ ID No.:102), A302GR (SEQ ID No.:103), A414A (SEQ ID No.:104), A468T (SEQ ID No.:105), A489A (SEQ ID No.:106), A502C (SEQ ID No.:107), A538TG (SEQ ID No.:108), BL39R (SEQ ID No.:109), BL50 (SEQ ID No.:110), BL77 (SEQ ID No.:111), BL272A (SEQ ID No.:112), BL263T (SEQ ID No.:113), BL527A (SEQ ID No.:114), BL570GT (SEQ ID No.:115), RA-2 (SEQ ID No.:116), RA-41 (SEQ ID No.:117), RB-28 (SEQ ID No.:118), 201g1 (SEQ ID No.:119), C206gR (SEQ ID

No.:120), R341A (SEQ ID No.:121), R343g3 (SEQ ID No.:122), 353TCC (SEQ ID No.:123), 361T1 (SEQ ID No.:124), 361T368g (SEQ ID No.:125), 361T368T1 (SEQ ID No.:126), 369C (SEQ ID No.:127), 387g1 (SEQ ID No.:128), 526AC2 (SEQ ID No.:129), 538gAC (SEQ ID No.:130), complementary strands thereof and nucleic acids which comprises one to several bases are deleted from or added to the end of them.

11. A primer used for a typing method of the HLA class I alleles, which is selected from the group consisting of BASF-1 (SEQ ID No.:88), BASR-1 (SEQ ID No.:89), CGA011 (SEQ ID No.:90), CGA012 (SEQ ID No.:91), AIn3-66C (SEQ ID No.:92), 5BCIn37-34C (SEQ ID No.:96), 5BCIn37-24g (SEQ ID No.:97) and BCIn37-34g2 (SEQ ID No.:99).

12. A kit for typing of the HLA class I alleles, which is used for the method claimed in any one of claims 1 to 9.

13. A reagent for typing of the HLA class I alleles, which is used for the method claimed in any one of claims 1 to 9.

14. A kit for typing of the HLA class I alleles, which comprises the DNA probe claimed in claim 10.

15. A reagent for typing of the HLA class I alleles, which comprises the probe claimed in claim 10.

16. A kit for typing of the HLA class I alleles, which comprises the primer claimed in claim 11.

17. A reagent for typing of the HLA class I alleles, which comprises the primer claimed in claim 11.

18.(Added) A method for detecting a specific base sequence, wherein hybridization is performed in a hybridization buffer containing 10% to 25% formamide, at 32°C to 42°C, using a probe of 14 to 24 or more of bases.

19.(Added) The method claimed in claim 18, wherein the hybridization buffer

contains 0.25M di-sodium hydrogenphosphate, 7% sodium dodecyl sulfate, 1% bovine serum albumin, 0.03M phosphoric acid, 0.5M ethylenediaminetetraacetic acid and 10% to 25% formamide.

20.(Added) The method claimed in claim 18 or 19, wherein the temperature for washing after the hybridization is room temperature.

21.(Added) The method claimed in any one of claims 18 to 20, wherein the probes are hybridized with amplified products by the PCR method.

22.(Added) The method claimed claimed in claim 21, wherein at least one of the primer pair is labeled.

23.(Added) The method claimed in any one of claims 18 to 22, wherein nucleic acids are hybridized with the probes immobilized on a support.

24.(Added) The method claimed in any one of claims 21 to 23, which comprises hybridizing the amplified products obtained by the PCR method with the immobilized DNA probes, adding an enzyme-conjugate which specifically bonds to a label of the amplified products thereto at the same time or after the hybridization, and adding a chromogenic substrate, a luminescent substrate or a fluorescent substrate to the mixture, to detect as signals whether or not the amplified products are hybridized with the immobilized DNA probes.

25.(Added) The method claimed in claim 24, whrein the label is a biotin and the enzyme-conjugate is an enzyme-conjugated streptavidin.